REMARKS

The Present Invention

The present invention is directed to methods and compositions for preparation of mutant high alkaline proteases and mutant alkalophilic *Bacillus* strains which produce only the mutant high alkaline protease and not the corresponding indigenous protease. The present invention is further directed to a detergent composition comprising as an active ingredient one or more high alkaline proteases and a laundry process employing the detergent composition.

The Pending Claims

Prior to entry of the above amendments, Claims 4-7, 9-16, 19, and 23-29 are pending. Claims 23, 4-7, 9-11, 26 and 28 are directed to methods for production of a mutant high alkaline protease; Claims 12-13 and 27 are directed to a method of obtaining an alkalophilic *Bacillus* strain having a reduced extracellular alkaline protease level; Claims 14-16 and 29 are directed to an alkalophilic *Bacillus* strain producing a mutant high alkaline protease; Claim 17 is directed to mutant high alkaline protease; Claim 19 is directed to a detergent composition comprising as an active ingredient high alkaline protease. Claim 24 is directed to a method of preparing a detergent composition comprising high alkaline protease as an active ingredient. Claim 25 is directed to a method of processing laundry with the claimed detergent composition.

The Office Action

The specification is objected to and Claims 4-7, 9-16, 19, and 23-29 are rejected under 35 U.S.C. 112, first paragraph, as failing to provide an enabling disclosure: specification is enabling only for claims limited to alkalophilic asporogenic Bacillus novo species PB92 of minimal natural extracellular protease level, transformed with a mutant B. novo PB92 alkaline protease; the specification is not properly enabled for Claims 4, 13-16 for any "derivative thereof" of a Bacillus novo species PB92; Claims 12 (and dependent Claims 13-16) and 27 (and dependent Claim 14) are rejected as unenabled by the specification for reasons previously made of record; Claim 29 is rejected as unenabled by the

specification for the host strain to be "substantially incapable of reversion"; and Claim 9 is rejected for being dependent from a rejected claim.

Claims 16, 19 and 23-26 are rejected under 35 U.S.C. § 112, second paragraph, as being indefinite: Claim 16 in its recitation of "or illegitimate recombination"; Claims 19 and 24-25 in its recitation of "one or more"; and Claims 23 and 26 for lack of reciting a "recovery step."

The Amendment filed September 7, 1993, is objected to under 35 U.S.C. § 132 for introducing new matter into the specification.

Claims 9, 19 and 23-26 are rejected under 35 U.S.C. § 112, first paragraph, as unenabled for new matter under 35 U.S.C. § 132 from the Amendment filed September 7, 1993.

The specification is objected to under 35 U.S.C. § 112, first paragraph, as unenabled for new matter under 35 U.S.C. § 132 from the Amendment filed September 7, 1993.

The Amendment filed November 22, 1994, is objected to under 35 U.S.C. § 132 because it introduces new matter into the specification.

The specification is objected to under 35 U.S.C. § 112, first paragraph, as unenabled for new matter under 35 U.S.C. § 132 from the Amendment filed November 22, 1994.

Claim 28 is rejected under 35 U.S.C. § 112, first paragraph, as unenabled for new matter under 35 U.S.C. § 132 from the Amendment filed November 22, 1994.

Claims 4-7, 9-16, 19, and 23-29 are rejected under 35 U.S.C. § 103 as being unpatentable over Fahnestock et al. and Estell et al., in view of TeNijenhuis and Suggs et al. for reasons previously of record.

Amendments

Claims 16 is cancelled to overcome a rejection.

Claims 9, 19 and 23-26 are amended to remove the phrase "exhibiting altered protease activity".

Claims 12 and 27 are amended to indicate that the transformants are grown under "growth conditions to which the replication function of said cloning vector is sensitive" and that the isolation step of the invention is accomplished by identifying those transformants

comprising the cloning vector having an "inactivated replication function" and having "no detectable extracellular high alkaline protease". Support for the amendment is found in the original claims and, for example, in the specification on page 8, line 25 through page 9, line 26.

Claims 19, 24, and 25 are further amended to indicate that the detergent compositions comprise "at least one of a mutant protease which has been prepared" according to independent Claim 23. Support for the amendment is found in the original Claim 23 and in the specification, for example, on page 12, lines 8-21, and page 15, lines 1-19.

Claims 23 and 26 are further amended recite a recovery step. Support is found in the specification, for example, on page 15, lines 1-19.

No new matter has been added by any of the amendments and the Examiner is respectfully requested to enter them.

For the Examiner's convenience, all pending claims, including amended and non-amended claims, are attached herewith as Appendix A.

Response to the Objections and Rejections

In the response that follows, the Examiner's individual objections and rejections are provided in full text, as identified by indented small bold print, followed by Applicants response.

35 U.S.C. § 112, first paragraph

The specification is objected to and Claims 4-7, 9-16, 19, and 23-29 are rejected under 35 U.S.C. 112, first paragraph, as failing to provide an enabling disclosure. The Examiner bases this rejection on the assertion that as the disclosure is enabling only for claims limited to methods of producing an alkalophilic asporogenic Bacillus novo species PB92 of minimal natural extracellular protease level, transformed with a B. novo PB92 alkaline protease which has been mutated as described in the specification. See M.P.E.P. §§ 706.03(n) and 706.03(z).

Applicants' arguments concerning the mutation of the high alkaline protease gene have been deemed persuasive. Applicants have convincingly demonstrated that the mutation to the protease per se is not the significant part of the application and invention, as stated, for example, at the sentence bridging pages 12-13.

The claims are not properly enabled for the recitation of any "mutant high alkaline protease", and any "alkalophilic Bacillus strain". Applicants have stated that the strain PB92 has been used merely as an example, and that the specificati n provides enablement for the use f ther types f these strains, and f r other "mutant high alkaline proteases".

Applicants further state that techniques f r such are "routine and require no inventive skill r undue experimentation" (pg. 7, response f 9-7-93). This is not deemed persuasive f r the reasons f record. Primarily, the specification has not provided pertinent inf rmation regarding any other "high alkaline protease" gene, nor any appropriate Bacillus strain that would satisfy the requirements of the invention. This fact is important, as the claims are not commensurate in scope with the specification and its enablement. This information is essential to the function of the claimed invention, and the essential material may not be improperly incorporated into the specification, and does not find support within the teachings of the specification. There is no teaching or reasonable expectation provided that the one skilled in the art would be able to utilize the teachings provided for any other systems/genes, or even that there is a problem with any other source such that the instant invention would be applicable. Absent this knowledge, one skilled in the art is left with an undue amount of experimentation, due to the breadth of the claims, in order to attempt to determine what other Bacilli or proteases would be useful in the instant invention, and then further attempt to find the gene and apply the principles taught herein. Thus, one skilled in the art would in no way be enabled to practice the claimed invention with any such gene or strain other than the enabled Bacillus PB92.

The objection to the specification and rejection to Claims 4-7, 9-16 and 23-29 are respectfully overcome in view of the current amendments and remarks that follow.

Applicants respectfully submit that the Examiner's primary argument for maintaining an enablement rejection that "the specification has not provided pertinent information regarding any other 'high alkaline protease' gene, nor any appropriate *Bacillus* strain that would satisfy the requirements of the invention" misses the point of the enablement requirement. The purpose of the specification is not to prove completion of every embodiment of the invention, but to teach one of ordinary skill in the art how to make and use the invention. The Patent and Trademark Office has established that an "example" is to "teach" the invention to a worker skilled in the art to let him carry it out, and not to "prove" a reduction to practice of every embodiment of the invention. In re Borkaowski, 422 F.2d 904, 164 U.S.P.Q. 642 (C.C.P.A. 1970), In re Wakefield, 422 F.2d 897, 164 U.S.O.Q. 642 (C.C.P.A. 1970), In re Marzocchi, 430 F.2d 220, 169 U.S.P.Q. 367 (C.C.P.A. 1971). This line of reasoning is also followed in the Federal Circuit. Atlas Powder Co. v. E.I. duPont de Nemours & Co., 750 F.2d 1569, 224 U.S.P.Q. 409 (Fed. Cir. 1984). The Patent Office has continued to apply this well settled issue that claims need not be limited to exemplification or preferred embodiments in order to satisfy enablement requirements. *Ex parte Gould*, 6 U.S.P.Q.2d 1680 (B.P.A.I. 1987). Indeed, to provide effective incentive,

claims must adequately provide coverage of embodiments to which Applicants are entitled. As stated by the *Goffe* court:

> "To demand that the first to disclose shall limit his claims to what he has found will work or to materials which meet guidelines specified for "preferred" materials in a process such as the case herein involved would not serve the constitutional purpose of promoting progress in the useful arts." *In re Goffe*, 542 F.2d. 564, 191 U.S.P.Q. 426 (1976).

Applicants stress that practicing the invention as claimed does not rest on molecular information of a particular alkaline protease gene or alkalophilic and/or asporogenic Bacillus strain. On the contrary, it depends on the ability to make and use Applicants' invention, the *inventive concept* of which was tested and demonstrated to work as exemplified by the expression of several mutant high alkaline proteases in various *Bacillus* novo species PB92 and PBT110-derived mutants. A key inventive concept of the instantly claimed invention is that mutant high alkaline proteases can be produced efficiently in the absence of indigenous high alkaline protease by using alkalophilic and/or asporogenous *Bacillus* strains comprising an indigenous high alkaline protease gene lacking its entire coding region that is substantially incapable of reversion back to wild-type. Again, Applicants need not prove a reduction to practice of every embodiment of the invention (see case law *supra*). Simply because all possibly useful *Bacillus* strains and genes encoding extracellular high alkaline proteases are unknown or not cloned does not mean that Applicants are limited to what they have exemplified. If that were true, others could with impunity use Applicants' inventive concept simply by substituting another protease gene as the target gene for removal, substituting a *Bacillus* other than PB92 or PBT110 as the expression host. Applicants have shown that their invention is operable as claimed and therefore is entitled to their generic claims.

As stated in previous responses of record, the cited MPEP sections do not support the present rejection. Section 706.03(n) simply requires *correspondence* between the language of the specification and the language of the claims, and it is not disputed that this requirement is met in the present application. Section 706.03(z) requires that the recitation of a *genus* of compounds in a claim (e.g., *Bacillus*) be supported by the *disclosure* of a sufficient number of *species* in the specification (e.g., PB92, PBT110, PBT110-INTS, PBT125, PBT126). It is

not a requirement for any number of working examples. It is respectfully submitted that the extensive disclosure of the PB92 and the PBT110-derived mutants in the specification at pages 12-18 is more than adequate to support the genus of *Bacillus* recited in the pending claims.

The question of enablement therefore rest on whether the specification adequately teaches the *concept* of employing high alkaline protease and *Bacillus* strains other than that exemplified by *Bacillus* novo species PB92 and PBT110. There is, however, no requirement in 35 U.S.C. § 112 or anywhere else in the patent law that a specification must convince persons skilled in the art that the assertions in the specification are correct. In examining a patent application, the Patent Office is required to assume that the specification complies with the enablement provisions of § 112 unless it has "acceptable evidence or reasoning" to suggest otherwise. *Gould v. Mossinghoff*, 229, U.S.P.Q. 1 (D.C. 1985). The PTO must therefore provide reasons supported by the record as a whole as to why the specification is not enabling; then and only then does the burden shift to the Applicant. Thus, in order for the Examiner's claim of nonenablement to stand in the face of Applicants' teachings in the specification, he must provide "acceptable evidence or reasoning" that other high alkaline genes and alkalophilic/asporogenic Bacillus strains would not work in accordance with the invention as claimed. It is respectfully submitted that the Examiner has failed to do so.

Furthermore, in contrast to the Examiner's premise for maintaining the objection and rejection, the specification does provide pertinent information for practicing the invention claimed regarding "high alkaline protease" genes and appropriate *Bacillus* strains other than those from *Bacillus* novo species PB92.

First, mutant high alkaline proteases are well known in the art and the specification provides examples of mutant high alkaline proteases on page 12, line 38 through page 13, line 3 in the Examples on page 23, lines 19-23 and in Table 1 on page 29. Examples of alkalophilic protease mutants cloned by Applicants include M216Q, M216S, S160D, N212D as provided on page 20 of the specification. The specification provides detailed procedures for producing mutant protease genes on page 21, line 32 through page 23, line 1.

Significantly, success in practicing the invention, whether molecular information is available or not, is evaluated by methods for selecting and characterizing protease negative strains as provided on pg. 17 and methods for evaluating protease activity as provided on pg. 28 line 27 - pg. 29 line 45. As noted above by Applicants, knowledge of protease gene structure before or after mutagenesis and the effect of gene mutation on protease activity is not essential to practice the instant invention. The mutant high alkaline protease as claimed is evaluated not by any knowledge of gene structure, but simply by determining whether the mutant protease exhibits altered protease activity (pg. 28, lines 27 - pg. 29, lines 12-16) or maintains the capacity to degrade substrate (pg. 13, lines 10-13).

From the teaching of the present application, one of ordinary skill in the art would understand that a *Bacillus* protease gene could be deleted by introducing a vector having the 5' and 3' flanking regions but not the coding region of the protease gene. For example, the skilled artisan could easily determine the termini of a test protease gene in a plasmid by using standard restriction enzyme mapping techniques. Once the protease gene termini are determined, a restriction enzyme could be chosen that removes the test protease gene sequence from the plasmid, but leaves the termini within the plasmid. Appropriate starting vectors and routine methods for manipulating DNA fragments between plasmids are disclosed pp. 15-16. Applicants urge that these techniques are routine and require no inventive skill or undue experimentation. Once a plasmid is made that comprises the termini of the test protease gene, it can be used to transform a *Bacillus* host (i.e., PB92), resulting in integration and inactivation of the host protease gene (pp. 16-17). The original plasmid comprising the test protease gene can be mutated by digestion with another restriction enzyme and used to transform the mutant *Bacillus* host in order to produce mutant protease (pg. 25-27).

Second, this invention is exemplified using *Bacillus* novo species PB92 and PBT110-derived mutants, but one of ordinary skill in the art would recognize that the invention could be practiced with any suitable alkalophilic and/or asporogenic strain of *Bacillus*. The definition for alkalophilic *Bacilli* is given in the specification on page 10, lines 19-33. Although not taxonomically well classified, these *Bacillus* species are well known in the art.

For example, the specification refers to the *Bacillus* novo species PB92 strain as an example of an alkalophilic *Bacillus* host strain and discloses other alkalophilic Bacillus strains that can be used for protease production (pg. 12 lines 8-21). The specification notes that the claimed methods find use in the production of mutant serine proteases using protease negative strains of alkalophilic *Bacillus* (pg. 4, lines 32-35; sentence bridging pp. 6-7). DNA Transformation protocols needed for these strains have also been disclosed (pg. 14, lines 23-25).

The specification need not provide a detailed disclosure regarding all the possible *Bacillus* strains and proteases that could be used in the instant invention, since references to the art have been provided. The Examiner is respectfully reminded that the specification need not disclose and preferably omits that which is well known in the prior art, Hybritech Inc. v. Monoclonal Antibodies, Inc., 802 F.2d 1367, 231 USPQ 81 (Fed. Cir. 1986). Collectively, the specification has disclosed methods of using several *Bacillus* strains and proteases to produce modified *Bacilli* which produce mutant protease in the absence of indigenous protease(s). Applicants have therefore met their burden of showing that the specification is enabling under 35 U.S.C. § 112, first paragraph.

Accordingly, Applicants respectfully submit that the specification and claims are enabled and request the objection to the specification and rejection to Claims 4-7, 9-16, 19, and 23-29 under 35 U.S.C. 112, first paragraph, be withdrawn.

> **The specification is not properly enabled for claims to any "derivative thereof" of a Bacillus novo species PB92. Applicants state that passages on page 12 of the specification refer to known "derivatives", and that this would be enabling for the instant invention. The phrase "derivatives thereto", however, encompasses predetermined and random mutants of the strain, and progeny of the strain that may or may not contain that gene for the "mutant high alkaline protease" and/or a revertant strain with the indigenous gene. The specification does not properly teach nor describe to one skilled in the art these "derivatives", what specifically they entail, nor how to obtain and/or use such. Mere reference to other teachings, when this is a matter of essential material, without an instant and specific teaching as to how these would be applicable, is not sufficient. Thus, this results in undue experimentation for one skilled in the art to attempt to produce such without proper guidance from the specification.**

The rejection is respectfully traversed.

The Examiner's statement that "reference to other teachings, when this is a matter of essential material, without an instant and specific teaching as to how these would be

applicable, is not sufficient" is misguided as to his assertion of nonenablement. Essential material is that which is required to practice the invention as claimed (i.e. that which is required to describe, enable or provide the best mode of the invention claimed; MPEP § 608.01(p)(B)). Applicants have provided explicit examples which employ *Bacillus* novo species PB92 and the specification further teaches how to make derivatives using standard techniques known in the art (pg. 10, line 11 - pg. 12, line 7). And their is no question that the specification references other derivatives of *Bacillus* novo species PB92 (pg. 12, lines 8-21) which were obtained using procedures known to those in the art and described in the specification (pg. 10, line 11 - pg. 12, line 7). The Examiner is reminded that to substitute a more preferred embodiment into the example where the Applicants believe that preferred results will be obtained is all to the good of the public. M.P.E.P. § 608.01(p). Thus any issue as to essential material is factually moot.

As to the construction and isolation of non-exemplified derivatives of *Bacillus* novo species PB92, the fact that the specification teaches (pg. 10, line 11 - pg. 12, line 7) and provides specific working examples and construction of derivatives (e.g., Example 2) cannot be overlooked. It is these very examples and the specification in whole that one of ordinary skill is to use as a guide to make other derivatives. Applicants respectfully remind the Examiner that the test for determining what constitutes undue experimentation is not merely quantitative, since a considerable amount of experimentation is permissible if it is merely routine or if the specification in question provides a reasonable amount of guidance with respect to the direction in which the experimentation should proceed to enable the determination of how to practice a desired embodiment of the invention claimed. *Ex parte Forman*, 230 U.S.P.Q. 546 (B.P.A.I. 1986). The Federal Courts have upheld the Board's standard concerning experimentation noting that if the amount of experimentation is "reasonable," the specification is enabling. *Cross v. Iizuka*, 753 F.2d 1040, 224 USPQ 739 (Fed. Cir. 1985).

Importantly, the Examiner has not met his burden of showing by either scientific rational or specific example as to why the present specification could not be used to obtain and generate other derivatives. The Examiner's reference to the possibility of generating

"random mutants" and "revertants" is insufficient reasoning. It is well known in the art that derivatives of any given population of bacteria can contain a certain level of random mutants and/or revertants, which is expected. Further, predetermined mutants of a species can be made, as correctly noted by the Examiner, which aspect is absolutely taught and enabled by the specification. Although some situations can be envisioned where the invention might be inoperable, this is not an issue. It is not the purpose of a claim to exclude possible inoperative substances. *Ex parte Hradcosky*, 214 U.S.P.Q. 554 (PTO Bd. App. 1982); *Atlas Powder Co. v. E.I. duPont de Nemours & Co.*, 750 F.2d 1569, 224 U.S.P.Q. 409 (Fed. Cir. 1984).

In view of the above remarks, the Examiner is respectfully requested to withdraw the rejection.

> **The method of claims 12 and 27 are not properly enabled by the specification. This stands for the reasons of record, as applicant has not properly and specifically traversed the rejection. The claimed invention is not reflective of the method and "conditions whereby the replication function encoded by said vector is inactivated". It would require an inordinate amount of experimentation for one of ordinary skill to attempt to determine what and where the "replication function" of the vector is, its relationship to the rest of the invention, a method of "inactivating" such, and its possible effects upon the instant invention. Further, the specification is not enabling for any or all possible methods of "identifying" transformants with no detectable indigenous protease. The claims are not commensurate in scope with the enablement of the specification for such methods.**

The rejection is believed overcome in view of the amendments and the remarks that follow.

Claims 12 and 27 have been amended to clarify the methods and conditions under which the replication function encoded by the vector is inactivated as it relates to the rest of the invention. Specifically, the claims are amended to indicate that the transformants are grown under "growth conditions to which the replication function of said cloning vector is sensitive" such that the replication function encoded by the vector is inactivated. The claims are further amended to clarify that the growing step relates to the isolation step of the invention which is accomplished by identifying those transformants comprising the cloning vector having an "inactivated replication function and no detectable extracellular high alkaline protease".

As to the identification of the replication function, the vector need not contain a replication function (see specification pg. 14, lines 11-15). When it is desired to include a replication function, the specification provides several examples for guidance (pg. 8, lines 30-33; pg. 19, lines 34 - pg. 20, lines 14). Nor would it require an inordinate amount of experimentation for one of ordinary skill to determine what and where the "replication function" of the vector is. There are absolute standard methods known in the art for accomplishing this goal, such as Southern blotting, restriction enzyme mapping, or simple selection methods, especially for vectors such as plasmids (see, for example, specification pg. 9, line 3-26).

Further, it is not the purpose of a specification to detail or even provide the "possible effects" that might occur by some step of the claimed invention. All that's required is the specification teach the invention as discussed *supra*.

As to identifying transformants, Applicants believe the claim amendments overcome this aspect of the rejection. However, Applicants wish to clarify the record as to the Examiner's statement that the specification is not enabling for "any ... methods of identifying transformants." The specification does indeed provide methods for identifying transformants (pg. 8, line 25 - pg. 9, line 9 and the Examples). And as a matter of law, the specification is not required to expressly disclose "all possible methods" of identification. *Chrvat v. Commissioner of Patents*, 503 F.2d 138, 182 U.S.P.Q. 577, 587 (D.C. Cir. 1974)(It is not necessary to describe in the specification all possible forms in which a claimed limitation may be reduced to practice).

As noted above, all that's required for enablement purposes is that the specification provide sufficient guidance to practice the invention claimed. Applicants have done so and the Examiner has not met his burden of showing why other methods known in the art would not be commensurate with the instant specific examples or why other methods of identification, routinely used in this field, would not be compatible with the method of the invention as claimed. The Examiner is respectfully reminded that it is the specification which teaches the invention and not the claim.

In view of the amendments and above remarks, the Examiner is requested to withdraw the rejection.

New claim 29 is not pr perly enabled by the teachings f the specification f r the host strain to be "substantially incapable f reversion". The specification, at page 7, lines 16-17, teach that the invention is (preferably) a "non-reverting mutation". This clearly conflicts with the phrase "substantially incapable". Thus, ne skilled in th art has not been taught by the specification, nor is enabled f r, the production f such strains that are nly "substantially incapable" of reversion. Similarly, since this is the case, and the gene encoding the high alkaline protease has been deleted, then it follows that there should be no "indigenous" alkaline protease product. Thus, it also follows that such a strain could not be "substantially free" of an indigenous alkaline protease, but instead, only completely free. The teachings of the specification do not provide support for such a strain being "substantially free", nor does the specification teach one skilled in the art how to produce such a strain.

The rejection is traversed because to limit Applicants to preferred embodiments is contrary to the purpose of the patent process and would deny Applicants effective coverage of their invention.

A statement of preferability in the specification in no way limits a claim. The fact that the specification teaches that the invention is "preferably" a non-reverting mutation does not conflict with the phrase "substantially incapable" of reversion. Although the specification teaches non-reverting and deletion mutants free of detectable levels of an indigenous extracellular high alkaline protease, the Examiner is overlooking a well known fact of the art that low level reversion is a process of evolution which is unavoidable in all living organisms. Mere selection pressure or lack thereof, for example, is enough to enhance the process of reversion and mutation in many bacterial strains. It is also possible through other well known means to generate strains that have increased rates of reversion and/or are more prone to random mutation. For example, one of ordinary skill in the art could easily make strains that are not 100% "incapable of reversion" or 100% "free of indigenous high alkaline protease" by simply referring to the specification on page 9, lines 2-26; strains missing the entire coding region of the target gene and having higher potential for reversion can be constructed.

Finally, as a matter of law, Applicants use of the word "substantially" in their patent claims is perfectly acceptable in order to prevent avoidance of infringement by minor changes that do not effect the results sought to be accomplished. *C.E. Equipment Co. v. United States*, 17 Cl. Ct. 293, 13 U.S.P.Q. 2d 1363, 1368 (Cl. Ct. 1989); *Arvin industries, Inc. v. Berns Air Kning Corp.*, 525 F.2d 182, 188, U.S.P.Q. 49, 51 (7th Cir. 1975)(The word "substantially" is not to be interpreted to negate the meaning of the word it modifies).

In view of the above remarks, the Examiner is respectfully requested to withdraw the rejection.

It should be noted that applicants have pointed out, at page 17 f the response filed 11-22-94, that the Examiner has erred in rejecting claim number 9 under 35 USC 112, 1st paragraph. This is not deemed persuasive. Although the claim is specific with regard to the source of the protease gene, it has not overcome all the deficiencies specified above for the claim(s) from which it is dependent (ultimately claim 23). This can be easily seen, for example, if the limitations of claim 9 were to be included with the limitations of the claims it depends upon in one singular claim. Thus, it is specific (clearly enabled) for one aspect, but not all, and is still properly rejected.

For reasons of this record, Applicants respectfully submit that Claim 23 is now in condition for allowance along with its dependents, including Claim 9, and the Examiner is respectfully requested to withdraw the rejection.

35 U.S.C. § 112, second paragraph.

The Examiner rejected Claims 16, 19 and 23-26 under 35 U.S.C. § 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

Claim 16 recites deletion of the gene of claim 15 (ultimately dependent upon claim 12) "by homologous or illegitimate recombination". The independent claim 12 only recites the method by "homologous recombination", and thus claim 16 is indefinite in its improper limitation. Applicants have incorrectly stated that since the claim is now ultimately dependent from claim 27, the issue is resolved. This is not the case, as the claim is still dependent from either claim 12, 13 or 27.

The rejection is rendered moot by cancellation of Claim 16.

Claims 19 and 24-25 are indefinite in the recitation of the phrase "one or more" of the protease produced. Since only one (type of) protease is being produced in the independent claims, the claims are indefinite for the recitation of "one or more". This is maintained for the reasons of record.

The claims have been amended to indicate that the detergent compositions recited in Claims 19 and 24-25 comprise at least one of a mutant protease which has been produced according to independent Claim 23. Accordingly, the Examiner is respectfully requested to withdraw the rejection.

The method of claims 23 and 26 are indefinite, as it is a method for producing a protease by culturing the host strain, yet there is not "recovery step" involved for obtaining the protease from the host. Applicants state that a recovery step is not essential. This is not deemed persuasive, as again, this is a necessary step to the claimed invention, as the mere production of the protease in the strain can not result in the claimed protease being accessible, and thus possessing utility.

The rejection is believed overcome in view of the amendments to recited a recovery step. For the record, however, Applicants maintain that the claimed inventive method does not require a specific recovery step because the host or a culture of the host itself may serve as a delivery vehicle for the protease. The utility of using the host strain as delivery vehicle is clearly recoginized, for example, by avoiding an undue recovery step when such a step is deemed unnecessary. For example, whether a host culture is grown and/or applied under conditions which permit lysing and/or secretion, the protease is accessible. Thus, there is no requirement that the protease be "recovered" from the host to realize utility and the claim is accurate as presented.

In view of the amendments and above remarks, the Examiner is respectfully requested to withdraw the rejection.

35 U.S.C. § 132

> **The amendment filed 9-7-93 is objected to under 35 U.S.C. § 132 because it introduces new matter into the specification. 35 U.S.C. § 132 states that no amendment shall introduce new matter into the disclosure of the invention. The added material which is not supported by the original disclosure is as follows:**
>
> **[a protease] "exhibiting altered protease activity".**
>
> **This phraseology is not supported at page 29 or anywhere else in the specification. Page 29, at best, describes "altered" protease levels of production, but does not describe the proteases having different activity. Applicants response filed 11-22-94 has been considered but not deemed persuasive. It stated that since the specification, at page 29, reads "the specific activities of the protease mutants... were used to determine the production of protease...", then this must imply that the activity of the enzyme has changed (i.e. "exhibiting altered protease activity"). This is incorrect. A change in protease level (i.e. production) may indeed be measured by the amount of activity present (i.e. the amount of enzyme present reflected by its activity), but this in no way implies that the actual activity of the enzyme has been changed. The amount of total cellular protease activity level has changed, but not the activity of the enzyme itself. In other words, the enzyme still functions the same way, even though there is less of the enzyme.**
>
> **Applicant is still required to cancel the new matter in the response to this Office action.**

The rejection is believed overcome in view of the amendments.

In Applicants' response mailed November 17, 1994, the phrase "exhibiting altered protease activity" was cancelled from Claim 14 and retained in Claims 9, 19 and 23-26. However, Applicants do not agree that the phrase constitutes new matter. To limit Applicants only to those exact words or phrases found in the specification to describe and set

the boundaries in claiming their invention is clearly unwarranted and contrary to patent law. The function of the description requirement is to ensure that the inventor had possession of the specific subject matter later claimed by him as of the filing date of the application relied upon; how the specification accomplishes this is irrelevant. In particular, the claimed subject matter need not be described in *haec verba* to satisfy the description requirement. M.P.E.P. § 608.01(o). Nor does the application need to be absolutely descriptive of the claim limitations, but only so clearly that one of ordinary skill in the art would recognize from the disclosure that the Applicants invented the subject matter claimed, including such limitations. *In re Herschler*, 591 F.2d 693, 200 U.S.P.Q. 711, 717 (C.C.P.A. 1979).

For the record, the Examiner has misrepresented the language cited from page 29 of the specification. It is well known to those of ordinary skill in the art that "specific activity" is a recognized term in the field that refers to an enzyme's activity which absolutely depends on factors other than the enzyme's concentration, including for example, substrate specificity, cofactor requirements, pH optima and various other kinetic properties. Using the Examiner's own example to illustrate Applicants' point, two enzymes can be present in the same concentration and have very different specific activities depending on numerous factors, such as those discussed immediately above. Nevertheless, Applicants' reference to this section of the specification was merely one example as to how protease activity can be measured and compared to wild-type protease activity.

Applicants respectfully draw the Examiner's attention to specification page 26, lines 24-36, as yet another example where the evaluation of "protease activity" is described. Here the specification clearly outlines that mutant proteases of the invention can be characterized based on their biochemical properties such as "(K_{cat}, K_m) oxidation resistance, specific activity and wash performance", which properties absolutely reflect mutant high alkaline protease exhibiting protease activity which can be "altered" compared to wild-type as detected when measuring such specific properties. There can be no doubt that this aspect of the invention was clearly envisioned by the inventors and expressly made available in the instant application to any person of ordinary skill reading and practicing the invention claimed. The phrase "exhibiting altered protease activity" does not, therefore, introduce new matter.

To advance prosecution of the present case the Applicants have amended the claims to exclude the phrase "exhibiting altered protease activity." Accordingly, the Examiner is respectfully requested to withdraw the rejection.

35 U.S.C. § 112, first paragraph

> **The specification is objected to under 35 U.S.C. § 112, first paragraph, as the specification, as originally filed, does not provide support for the invention as is now claimed This is deemed necessary for the reasons recited immediately above.**

The objection to the specification is respectfully traversed for the reasons presented above in response to the new matter rejection.

> **Claims 9, 19 and 23-26 are rejected under 35 U.S.C. § 112, first paragraph, for the reasons set forth immediately above in the objection to the specification regarding the new matter (applicant has only cancelled such in claim 14).**

The rejection to Claims 9, 19 and 23-26 is respectfully traversed for the reasons presented above in response to the new matter rejection.

35 U.S.C. § 132

> **The amendment filed 11-22-94 is objected to under 35 U.S.C. § 132 because it introduces new matter into the specification. 35 U.S.C. § 132 states that no amendment shall introduce new matter into the disclosure of the invention. The added material which is not supported by the original disclosure is as follows:**
>
> **The phrases "minimal indigenous extracellular protease level", and "a specifically-mutated Bacillus novo PB92 alkaline protease" (underlining added for emphasis) in claim 28.**
>
> **Applicants, at page 8 of the response, have stated that since the Examiner "indicated as enabled" these phrases, that a claim quoting these phrases seemed proper. This is not. These phrases are merely quotes from the Office Action, in the Examiner's shorthand manner of expressing the enablement of the specification. Obviously, the specification deals with very low levels of expression product, but does not exactly mention "minimal indigenous" protease. Although the two might be grammatically interchangeable, this was not a suggestion or invitation to use this in the claims. This also applies to the phrase "specifically-mutated", which was a shorthand way of avoiding the listing of each mutation used or available to applicant.**
>
> **Applicant is required to cancel the new matter in the response to this Office Action.**

The rejection is respectfully traversed for the following reasons.

The terms "minimal indigenous extracellular protease level" and "a specifically-mutated Bacillus novo PB92 alkaline protease" do not introduce new matter (emphasis by the Examiner reiterated). The phrase "specifically-mutated" is expressly found in the

↑ No spec. says
Native thermomyces
Not this PB92.

specification and carries with it the definition as used in the pertinent art (see specification page 10, line 5, for example). The term "indigenous" is clearly found in the specification and embodies the very same definition as recognized in the art (see specification page 7, line 13, for example). Similarly, the phrase "minimal indigenous" is clearly descriptive of the invention disclosed. This is evidenced by the Examiner's own statement regarding Applicants' use of the terms provided in the Examiner's responses. As stated by the Examiner in the most recent Office Action: "These phrases are merely quotes from the Office Action, in the Examiner's shorthand manner of expressing the enablement of the specification" (emphasis added). If "the enablement of the invention" is expressly communicated by the shorthand terms used by the Examiner as he understands the invention as communicated by the specification, use of the very same terms in the present claims cannot constitute new subject matter because enablement and subject matter requirements in this instance are inseparable. Again, how the specification describes the invention is not subject to limitation by specific words or phraseology. See case law *supra* and M.P.E.P. § 608.01(o).

Accordingly, the specification is clear in its description of the invention, including the those claim limitations not expressly quoted by the specification, and the Examiner is respectfully requested to withdraw the rejection.

35 U.S.C. § 112, first paragraph

> **The specification is objected to under 35 U.S.C. § 112, first paragraph, as the specification, as originally filed, does not provide support for the invention as is now claimed. This is deemed necessary for the reasons recited immediately above.**

The objection to the specification is respectfully traversed for the reasons stated above.

> **Claim 28 is rejected under 35 U.S.C. § 112, first paragraph, for the reasons set forth immediately above in the objection to the specification.**

The rejection to Claim 28 is respectfully traversed for the reasons stated above.

35 U.S.C. § 103

Claims 4-7, 9-16, 19, and 23-29 are rejected under 35 U.S.C. § 103 as being unpatentable over Fahnestock et al. and Estell et al., in view f TeNijenhuis and Suggs et al. The references and rejection are herein incorporated as cited in a previous Office Action.

Applicants' arguments filed in response to this rejection have been fully considered but they are not deemed to be persuasive. Arguments submitted 11-22-94 do not substantially differ from those already presented previously, and thus are not deemed persuasive for the reasons of record.

The rejection is respectfully traversed in part because of Applicants' previous responses of record and further in view of the current amendments and remarks that follow.

Applicants response mailed November 17, 1994 provided amendments, raised new points and expanded on former arguments to clarify and further differentiate the claimed invention from the cited art. The Examiner, however, has responded by broadly stating that none of Applicants' arguments are deemed persuasive "for reasons of record", which Applicants must assume as meaning all reasons of record. In order for Applicants to be able to fully consider and respond to the Examiner's rejection, the Examiner is earnestly requested to fully explain why each point of Applicants' response has been deemed non-persuasive. Particularly those amendments and points which relate to aspects of the invention not addressed by the Examiner's rejection.

In the instant response, Applicants faithfully reiterate these points and present to the Examiner further reasons as to why Fahnestock et al. and Estell et al., when taken in view of the secondary references of TeNijenhuis et al. and Suggs et al., would not have rendered the presently claimed invention obvious at the time the invention was made.

In substantiating a rejection under 35 U.S.C. § 103, the proper test for obviousness is whether the cited references, taken as a whole, would have suggested the invention of one of ordinary skill in the art. Milliken Research Corp. v. Dan River, Inc., 739 F.2d 587, 222 U.S.P.Q. 571, 583 (Fed. Cir. 1984). Consideration must also be given to teachings in the references which would have led one skilled in the art away from the claimed invention. Ashland Oil, Inc. v. Delta Resins & Refractories, Inc., 776 F.2d 281, 227 U.S.P.Q. 657 (Fed. Cir. 1985). And it is well settled that the Examiner is not free to pick and choose among the teachings of a reference to formulate an assertion of obviousness. In re Wesslau, 147 U.S.P.Q. 391 (C.C.P.A. 1965).

Moreover, to substantiate a holding of obviousness under 35 U.S.C. § 103, it is also impermissible to dismiss any surprising or unexpectedly useful properties of an invention. *See Perkin-Elmer Corp. v. Computer Vision Corp.* 732 Fed.2d 888, 221 USPQ 669 (Fed. Cir. 1984); *In re Fine*, 837 Fed2d 1071, 5 USPQ2d 1596 (Fed. Cir. 1988); and *In re Wright*, 848 Fed.2d 1216, 6 USPQ2d 1959 (Fed. Cir. 1988).

As previously detailed, a key inventive concept of the instantly claimed invention is that alkalophilic and/or asporogenous strains of *Bacillus* can be constructed which permit efficient expression of mutant high alkaline protease in the virtual absence of detectable levels of indigenous extracellular protease by deleting the entire coding region of a gene encoding indigenous extracellular high alkaline protease.

Based on the above cited case law, the Examiner is respectfully reminded that before obviousness of Applicants' inventive concept may be established, the Examiner must show that there is either a teaching, suggestion or compelling motivation based on sound scientific principles in the cited prior art to produce the claimed invention. As a threshold matter, the references must be taken for what they teach or suggest in whole and the Examiner is reminded that he is not free to reconstruct Applicants' invention in piecemeal hindsight. See case law *supra* and MPEP § 707.07(g). Furthermore, it is impermissible to dismiss any unexpected results or properties of the claimed methods and compositions produced therefrom.

With the above aspects of the invention and requirements for establishing obviousness in mind, Applicants now discuss the pertinent points of the cited references and why they would have failed to render obvious Applicants' claimed invention at the time the invention was made.

Primary References of Fahnestock et al. and Estell et al.

Fahnestock et al. and Estell et al. teach methods for obtaining non-alkalophilic *Bacillus* strains which contain an indigenous extracellular non-high alkaline protease whose expression is thought to be inactivated by (1) insertion mutagenesis using an antibiotic marker gene or (2) partial deletion of the coding region by chemical mutagenesis,

respectively. The non-alkalophilic *Bacillus* strains of Estell et al. and Fahnestock et al. are reported to express a protein encoded by an exogenously introduced gene in the absence of detectable levels of an extracellular neutral protease, but only reduced levels of extracellular alkaline protease, which the authors report are due to the presence of indigenous extracellular alkaline protease genes other than the mutated target protease gene.

In contrast, Applicants' independent Claims 12, 23, 26, 27 and 29 are directed to compositions and methods employing alkalophilic and/or asporogenic strains of *Bacilli* which lack at least the entire coding region of an indigenous extracellular high alkaline protease gene. Further, independent Claim 28 is directed to a method for producing a mutant high alkaline protease in an alkalophilic asporogenic *Bacillus* strain novo species PB92 of minimal indigenous extracellular protease. The methods and strains of the instant invention are surprisingly useful for the efficient production of mutant high alkaline protease in the complete absence (Claims 12, 27 and dependents) or substantially free (Claims 23, 26, 28, 29 and dependents) of detectable levels of any indigenous extracellular high alkaline protease. Moreover, Applicants' method provides an indigenous high alkaline protease gene which is virtually incapable of reversion back to wild-type or some other subrevertant because the entire coding region is deleted; these aspects are limitations of Claims 23, 29 and dependents. The cited references fail to teach or suggest any of the above aspects of the invention as claimed for the following reasons.

The methods of Fahnestock et al. and Estell et al. are exemplified by the use of *B. subtilis*, which strain is not alkalophilic. That alkalophilic *Bacilli* were simply known to exist in the art (e.g. TeNijenhuis et al.) is of no consequence because the primary references specifically teach and suggest the use of their methods with non-alkalophilic *Bacilli*; there is no suggestion or incentive to attempt the methods of Fahnestock et al. and Estell et al. with any alkalophilic *Bacilli* and certainly no expectation that their methods might be applicable in any *Bacillus* strain other than those that are non-alkalophilic. Indeed, undermining the actual teachings of Fahnestock et al. and Estell et al., and thus making the Examiner's extrapolation from non-alkalophilic to alkalophilic *Bacillus* one requiring a leap of faith, were problems known in the art when *Bacilli* have been used to produce proteases (see specification page 1,

line 31 - page 2, lines 1-19), including the fact that special methods of transformation are required for alkalophilic *Bacilli* (see specification page 14, lines 23-25). Additionally, neither Estell et al. or Fahnestock et al. teach or suggest the production of any high alkaline protease much less mutant high alkaline protease; although subtilisin is an alkaline protease, it is not a high alkaline protease and the two possess different properties.

As to asporogenic strains of *Bacilli*, the Examiner has continually disregarded the fact that Estell et al. expressly teach away from using asporogenic strains of *Bacilli* for the production of any recombinant protein of interest. That Fahnestock et al. used asporogenic strains of *Bacilli* expressing chloramphenicol resistance from the CAT insert does not detract from the express teaching of Estell et al. Fahnestock et al. did not assay the level of CAT expression or attempt its recovery. Moreover, CAT is not a protease or an extracellular protein and thus in no way reflects the ability of such strain to produce any recombinant intra or extracellular protease. In fact, Fahnestock et al. failed to show expression of any protein other than CAT, much less alkaline protease, much less a high alkaline protease. This is important because if Fahnestock's strains were to be used for expression of any protein other than CAT, one of ordinary skill in the art would have been forced to turn to the teachings of Estell et al. and what was known in the art in order to avoid reliance on the speculative disclosure of Fahnestock et al. Thus, even at the time the primary references were made available to the public, one of ordinary skill in the art would not have expected the asporogenic non-alkalophilic strains of Fahnestock et al. to efficiently express an alkaline protease, and certainly not any high alkaline protease, because of the lack of any substantiating results in Fahnestock and particularly because of what was known in the art and expressly taught by Estell et al.

In contrast, Applicants unexpectedly demonstrated in the face of the opposing teachings of both Estell et al. and Fahnestock et al. that asporogenic strains of *Bacillus* could be used to efficiently express high alkaline protease in the absence of indigenous extracellular high alkaline protease. The above aspects of Applicants' inventive concept and unexpected results cannot therefore be obvious as a matter of law or sound scientific reasoning.

Furthermore, with reference to Applicants' methods of constructing alkalophilic and/or asporogenous *Bacillus* strains comprising an indigenous extracellular high alkaline protease gene lacking its entire coding region, neither Fahnestock et al. or Estell et al. would have rendered Applicants' method obvious, whether taken alone or in combination. The Examiner's assertion that Fahnestock et al.s' deletion and replacement steps by homologous recombination would have rendered Applicants' method obvious is misguided and out of context with the teachings of that reference. Fahnestock et al. teach that the inactivated protease gene is integrated into the chromosome by a "two-step insertion excision process" which occurs simultaneously in the same irreversible reaction: the so called "first step" results in tandem duplication in which one of two copies of the protease gene is unaltered; the so called "second step" resolves the duplication event in which the altered (CAT insertion mutant) or unaltered (indigenous) protease gene is retained (see Fahnestock et al., column 4, lines 17-35, and Figure 4). The reactions are inseparable and the outcome is either an inactive indigenous protease gene with tracks of coding region present that are capable of reversion back to wild-type or an intact active indigenous protease gene; this is a fact of scientific principle.

In no case does Fahnestock et al. obtain or suggest progeny which lack an indigenous protease gene missing the entire coding region, either altered or unaltered, and especially not a high alkaline extracellular protease. In order for Fahnestock et al's. method to work, recombination absolutely requires the replacement of the indigenous protease gene with one that contains an inactivating gene insert (e.g. the CAT gene insertion). It is apparent that if one would have been motivated to substitute in place of the CAT sequence a protease encoding sequence, the resulting progeny would have consisted of active protease genes only, which would have completely frustrated the invention of Fahnestock et al., i.e. the goal of eradicating protease expression. Even if the Examiner's reading of the reference is applied, the teachings of Fahnestock et al. at best would have directed one of ordinary skill in the art to insert a protease coding region within the indigenous protease sequence. This does not render obvious any of Applicants' claims which specifically requires that the entire coding region of the indigenous protease gene be removed. Estell et al. fails to remedy this and the other fatal deficiencies of the Fahnestock et al. reference.

The Examiner cites Estell et al. for teaching the partial deletion of the coding region of an indigenous extracellular protease gene and the obtention therefrom of strains that produce no detectable levels of neutral protease. He further states that it would have been obvious to delete the remainder of the coding region "for the mere assurance of complete success of no protease activity." The Examiner also cites the general field of genetics as teaching deletion of whole genes. This reading is impermissibly made only through hindsight analysis of Applicants' disclosure.

One of ordinary skill in the art would not have been motivated to delete the remainder of the coding region "for the mere assurance of complete success of no protease activity" whether taken in view of the general field of genetics and especially in view of Fahnestock et al. Establishment of motivation is not abstract and cannot be substantiated in hindsight. Instead, the motivation to go beyond the specific teachings of a reference must be compelled by logic and is always related to the properties that one skilled in the art would have expected the method and products produced therefrom to have.

Estell et al. and Fahnestock et al. do not provide the motivation to delete the entire coding region of an indigenous extracellular protease gene because one of ordinary skill in the art would not have expected such a step to provide any different results than those already obtained by their methods. Estell et al. and Fahnestock et al. teach and suggest that the target protease gene's activity is thought by the authors to be effectively destroyed by either insertion (e.g., CAT gene insertion) or partial deletion (e.g., chemical mutagenesis). Thus one of ordinary skill in the art would not have been motivated to delete the entire protease gene or its coding region because such a step would have been expected to be redundant and not expected to provide any improved qualities for producing any protein in the resultant *Bacillus* strain. This is further made apparent by Fahnestock et al. which specifically teaches away from further manipulation of the target indigenous protease gene. For example, Fahnestock et al. teaches that to further reduce extracellular protease activity, other indigenous extracellular protease genes are to be disrupted by their method of insertional mutation (Fahnestock et al. col. 4). There is no mention or suggestion in either Estell et al. or Fahnestock et al. to delete the entire coding region of any gene nor why it would be advantageous. Accordingly, one of ordinary skill in the would not have been

motivated to veer from the teachings of Fahnestock et al. and Estell et al. and thus would not have been motivated to attempt to delete the entire coding region of a target indigenous protease gene.

Secondary References of TeNijenhuis et al. and Suggs et al.

The TeNijenhuis et al. and Suggs et al. references add nothing to the primary references. As stated in previous responses to the Patent Office, TeNijenhuis et al. discloses the presence of indigenous alkaline protease in *Bacillus novo* species PB92 and cultivating a *Bacillus* strain PB92 in a nutrient medium, and then isolating the proteolytic enzyme formed from the fermentation broth. Suggs et al. teach isolation of cDNA encoding human ß2-microglobulin based on partial amino acid sequence data; there is no mention whatsoever of any bacterial protease. A generic method for cloning human cDNA based on partial amino acid sequence data (Suggs et al.) and the fermentation and partial purification of a bacterial protease enzyme (TeNijenhuis et al.) adds nothing to the teachings of Estell et al. and Fahnestock et al. that would have rendered obvious Applicants' inventive concept for reasons already discussed above.

As maintained by the Examiner, however, TeNijenhuis et al. disclose a purified high alkaline protease from *Bacillus* novo strain PB92 and that this protease can be produced in its originating host strain of *Bacillus* PB92 in "surprisingly high levels." He believes that such an observation would have made obvious the expression of a mutant high alkaline protease in a *Bacillus* PB92 host which had the coding region of a corresponding indigenous extracellular protease deleted. This inference is grossly misguided and clearly made in hindsight. Indeed, to reach this conclusion, the Examiner maintains that the *Bacillus* PB92 strain and partially purified protease of TeNijenhuis et al. when combined with the method of Suggs et al. would have made obvious the cloning of the gene if any portion of the amino acid sequence were known. TeNijenhuis et al. does not disclose any portion of amino acid sequence sufficient for the method of Suggs et al. to work and thus any combination of Suggs et al. and TeNijenhuis et al. by the Examiner's own reading is moot. The Examiner's combination of

In view of the above amendment and remarks, it is submitted that this application is now ready for allowance. Early notice to that effect is solicited. If in the opinion of the Examiner, a telephone conference would expedite the prosecution of the subject application, the Examiner is invited to call the undersigned at (415) 926-6205.

Respectfully submitted,

Dated: July 7, 1995

Barbara Rae-Venter, Ph.D.
Reg. No. 32,750

WEIL, GOTSHAL & MANGES
2882 Sand Hill Road, Suite 280
Menlo Park, CA 94025-7022
Telephone: (415) 926-6200
Facsimile: (415) 854-3713

BRV/JAB/jgd
SVFS01...:\03\48403\4230\0238\RSP4275L.010